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14. ABSTRACT

The overall objective of this proposal is to develop a durable cure for lethal prostate cancer through the elucidation of the role of cancer stem cells in the pathogenesis of the disease. During the past year, we have made the following significant findings/observations: i)3D culture of human prostate cancer cells with magnetic nanoparticles is not optimal for tumor initiation studies, ii) in vitro co-culture of human prostate cancer cells (established cell lines and primary patient samples) with human prostate fibroblasts hold promise as models of tumor initiation/cancer stem cell activity. We continue to optimize and validate our in vitro model of prostate cancer initiation to facilitate cancer stem cell discovery as well as drug targeting.

15. SUBJECT TERMS
Prostate cancer, stem cell, in vitro assay, cancer stem cell

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Introduction

The overarching goal of this proposal is to develop a durable cure for men with advanced prostate cancer through an improved understanding of the role of human prostate cancer stem cells in the pathogenesis of the disease. To this end, we have proposed the following specific aims: **1)** to identify and prospectively isolate prostate cancer stem cells from human prostate cancer tissue, **2)** to examine human prostate cancer cell lines, both primary and established, for cells that express cancer stem cell surface markers and the ability to determine therapy resistance *in vitro*, and **3)** to develop an *in vivo* model to assess human prostate cancer stem cell targeted therapy. The elucidation of the differential biology of cancer stem cells, versus the bulk population of cancer cells, has the potential to lead to the identification of novel therapeutic targets that aim to cripple the driving force behind lethal prostate cancer.

Body

Revised September 4, 2009 **Grant # W81XWH-08-1-0508**

Revised/new Statement of Work

Isolation and characterization of human prostate cancer stem cells

Research plan

Task 2a: *In vitro* examination of human prostate cancer cell lines, both primary and established, for cells that express cancer stem cell surface markers. (months 6-24)

As noted in our last annual report, we have faced challenges in readily obtaining rSVM as a feeder layer for in vitro assays as we have previously described (Silvers et al, 2010). In the past year, we have focused attention on two alternative strategies: magnetic nanoparticles and human prostate fibroblasts serving as a feeder later.

Magnetic nanoparticles

Souza et al previously have shown that magnetic nanoparticles are able to passively diffuse into live cells, in an inert manner, and are capable of inducing 3D in vitro cell growth in a magnetic field.[1] This technique is based on the cellular uptake and magnetic levitation of a bioinorganic hydrogel (phage, magnetic iron oxide (MIO) and gold nanoparticles; see Figure 1). Notably, this technique has been observed to be useful in cultivating human primary cancer cell lines that are difficult to maintain in traditional cell culture. We obtained the magnetic nanoparticle reagents from n3D Biosciences (Houston, Texas).

Figure 1. Magnetic Levitation Based Three-Dimensional Tissue Culture

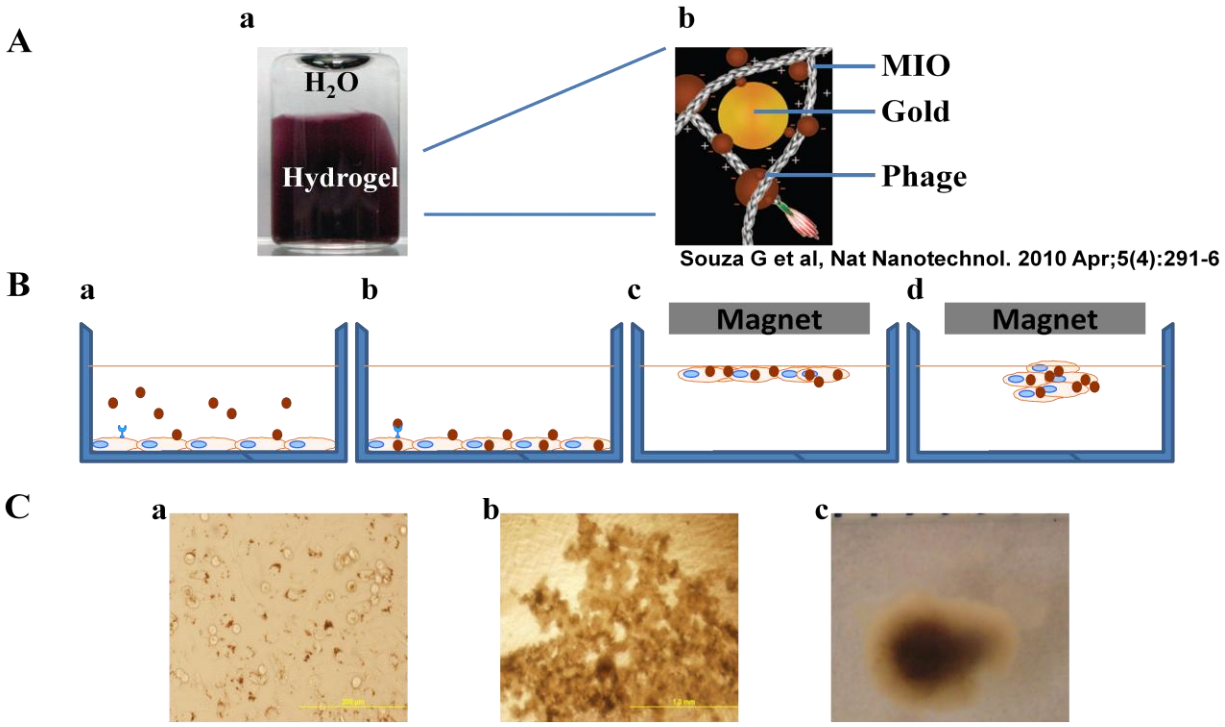

Figure 1. Magnetic Levitation Based Three-Dimensional Tissue Culture. A, Magnetic iron oxide-containing hydrogels. a, MIO-containing hydrogel in water. b, The scheme of hydrogel components with MIO, gold nanoparticles and phage. B. The strategy of cell levitation. a, Hydrogel is incubated with cells. b, hydrogel particles are uptaken by cells. c, magnetic pad raise cells to the air-medium interface. d, multicellular sphere structure forms. C. Prostate cancer cells were cultured with MIO. a, Brown colored hydrogel particle inside the cells after overnight incubation. b, 3 days after application of the magnetic force, cells levitated to the air-medium interface starting to form a three dimension structure. c, A sphere(LNCaP) like structure(~4mm in diameter) formed after 3 weeks culture.

Initially, we tested this technique with human prostate cancer cell lines. As shown in Figure 2, we were able to grow 3D clusters of LNCaP cells with this system that resembled 2D colonies with regards to PSA (+) and CD44 (-) expression.

Figure 2. Immunohistochemistry analysis of two dimensional and three dimensional culture with magnetic hydrogel

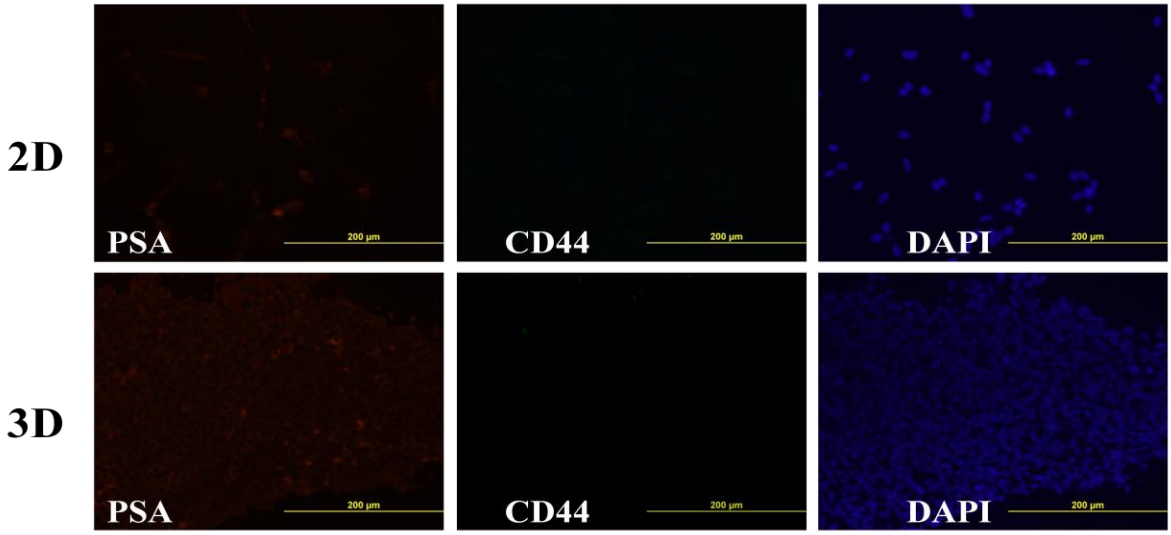

Figure 2. Immunohistochemistry analysis of two dimensional and three dimensional culture with magnetic hydrogel. Upper panels: LNCaP cells in the conventional two dimensional culture were fixed and Immunohistochemistry was performed with PSA antibody (red) and CD44 antibody (green) and DAPI (purple). Lower panels: LNCaP cells under three dimensional culture with magnetic hydrogel formed a sphere like structrue as showed in Figure 1, C(c). The sphere was paraffin-fixed and sectioned and after antigen retrieval, PSA antibody (red) and CD44 antibody (green) and DAPI (purple) were used for Immunohistochemistry.

We further evaluated this methodology with the xenograft prostate cancer cell line LAPC9, similar to what we had done before with rSVM (Figure 3).

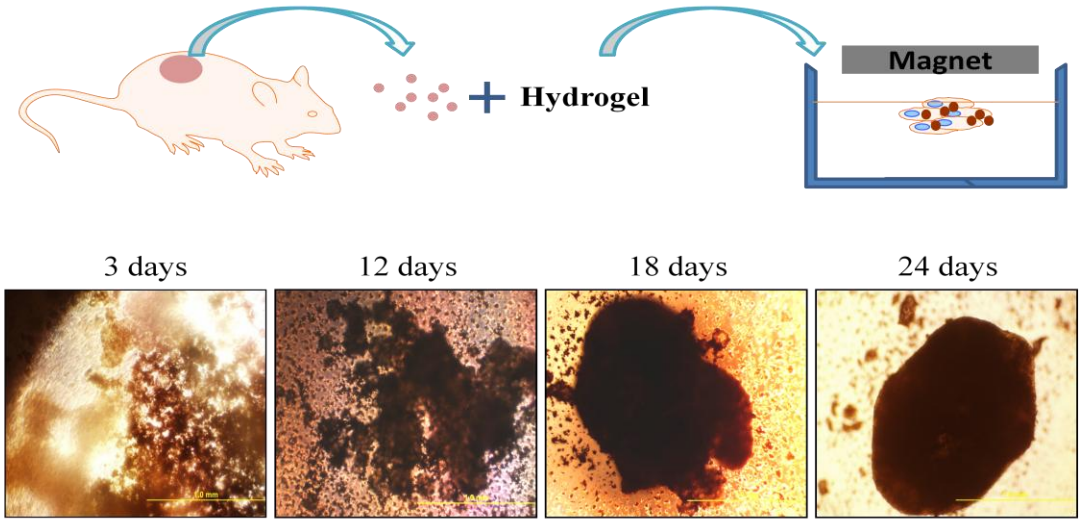

Figure 3. Three dimensional culture of xenograft prostate cancer cell line-LAPC9 with magnetic hydrogel. LAPC9 is a prostate xenograft cell line usually not growing in conventional two dimension conditions. Fresh LAPC9 tumor was dispersed into single cells and then incubated with MIO containing hydrogel, and the magnetic force was applied as showed in the upper panels. Cells levitated on the air-medium surface were analysed under the microscopy daily and the images at 3, 12, 18 and 24 days were taken as showed in the lower panels.

We then tested the capacity of this technique to grow 3D cultures from primary human prostate cancer patient samples (Figure 4). After 2-4 weeks, we observed 3D colony formation from 6 distinct patient samples; beyond 4 weeks the 3D structures began to break down and fragment. Single cells plated from these same samples in traditional 2D culture yielded no adherent epithelial cells (data not shown). We then attempted xenograft transplantation with matrigel in immunocompromised mice- none yielded viable tumor.

Figure 4. Three dimensional culture of fresh prostate cancer patient cells with magnetic hydrogel

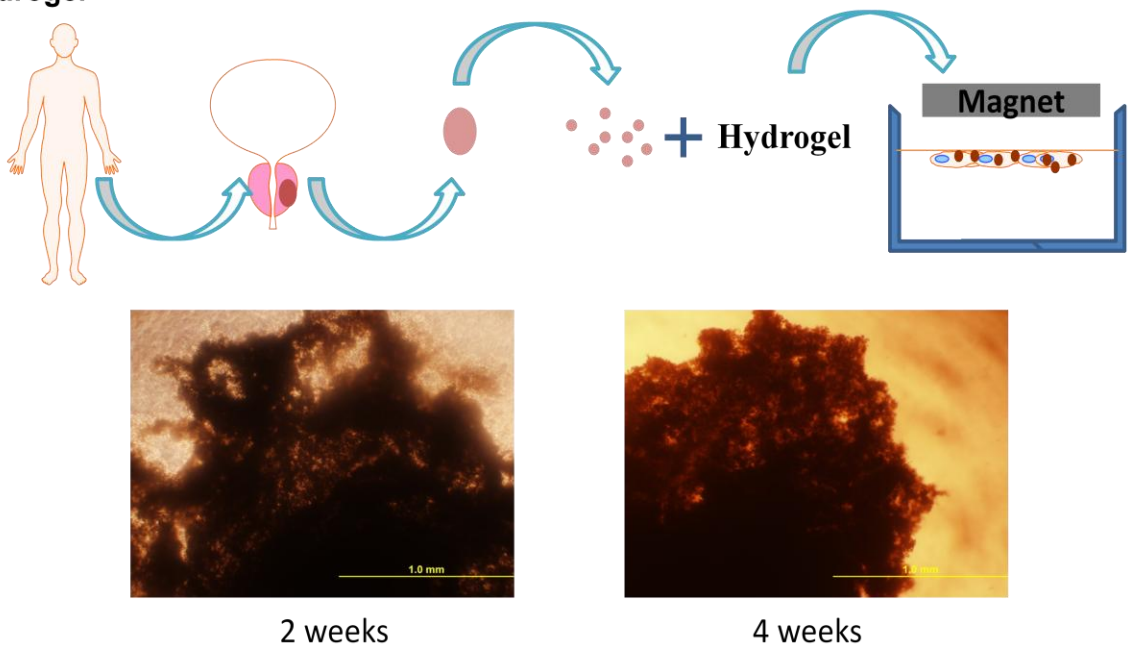

Figure 4. Three dimensional culture of fresh prostate cancer patient cells with magnetic hydrogel. Fresh prostate cancer patient samples were obtained in accordance with appropriate IRB protocols. Surgical resected prostate tissue was dispersed into single cells and then incubated with MIO containing hydrogel, then the magnetic force was applied as showed in the upper panels. Cells levitated on the air-medium surface were analyzed under the microscopy daily and the images at 2 weeks and 4 weeks were taken as showed in the lower panels.

Human prostate fibroblasts as a feeder layer

Through a collaboration with David Rowley, PhD at Baylor College of Medicine, we have obtained a human prostate fibroblast line (HPS 19I) from a 19 y/o motor vehicle accident victim. This line spontaneously immortalized and contains no gross chromosomal aberrations by spectral karyotype. Further, this line has been in culture for >2 years, has been passed >40 times and is non-tumorigenic. The Rowley lab has developed a co-culture system wherein 19I cells can co-mingle with prostate cancer epithelial cells to form 3D structures termed organoids (Figure 5). We have had initial success with cultivating prostate cancer lines in this system (Figures 6, 7).

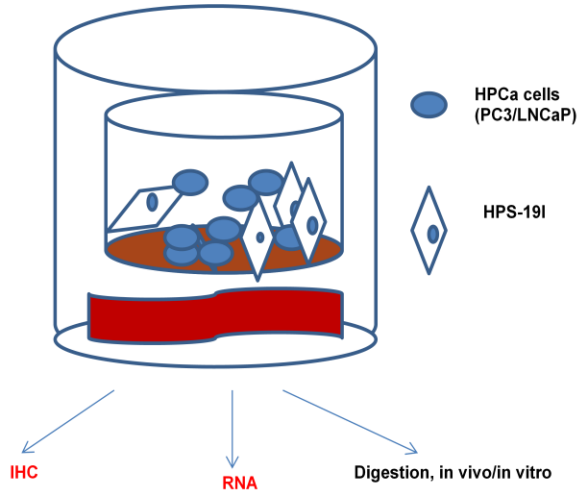

Figure 5. Stromal-epithelial co-culture system. In this system devised by the Rowley lab, epithelial cells (e.g., PC3, LNCaP) are mixed with HPS19I stromal cells in supportive media. A stirrer bar below helps circulate media in the chamber. Resultant 3D structures, organoids, can then be harvested for IHC, RNA or further studies.

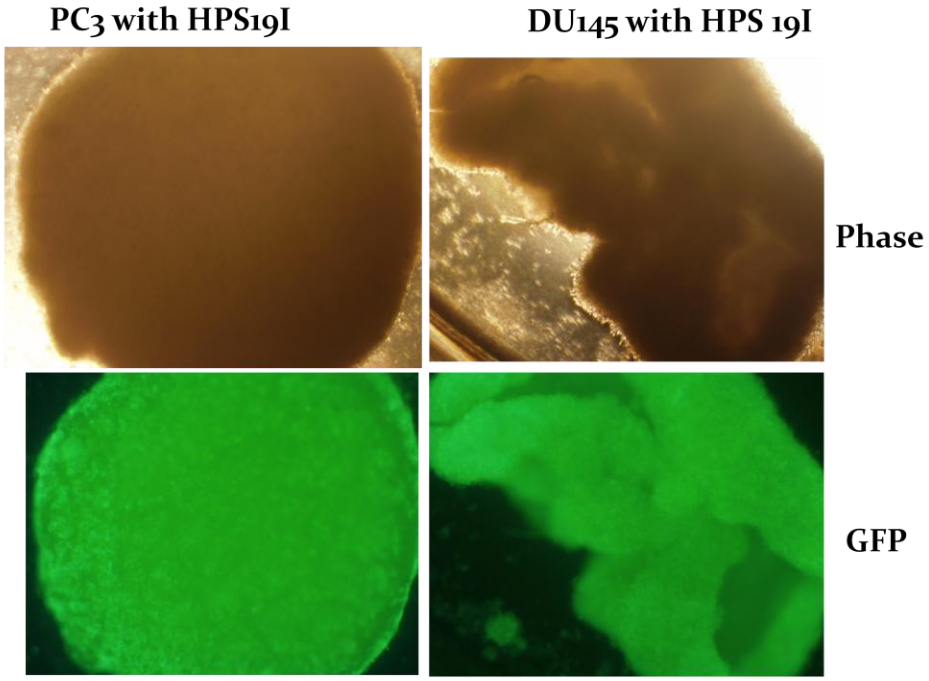

Figure 6. HPS191 and prostate cancer cell lines in vitro. After 2-3 weeks, organoids composed of stromal and epithelial cells (i.e., PC3 or DU145 transduced with GFP) are visible.

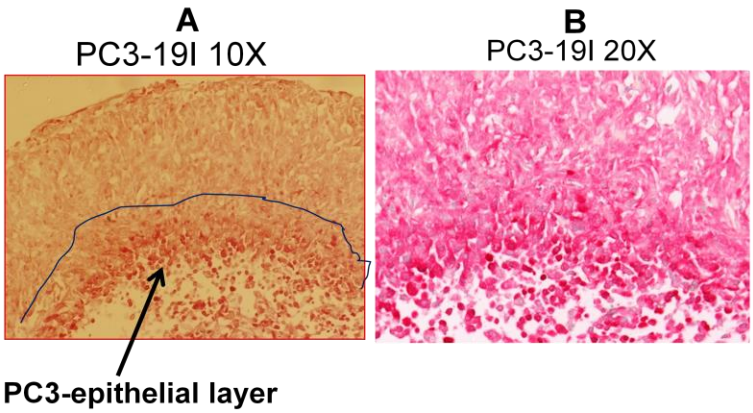

Figure 7. HPS191 and prostate cancer cell line organoids. After 2-3 weeks of co-culture, organoids form with stromal cells on the outside and epithelial cells on the inside. In panel A, line marks stromal-epithelial border. Panel B shows same border at higher magnification (H&E).

We are currently employing this stromal culture technique with primary patient samples with the goal of assaying resultant organoids for prostate cancer markers (IHC) and tumor initiation (xenograft studies).

Task 2b: Assessment of the ability of human prostate cancer cells that possess cancer stem cell surface antigen expression to determine therapy resistance *in vitro*. (months 14-28)

As noted, due to issues with developing an in vitro assay of tumor initiation no work on this sub-aim has been performed to date.

Task 3: Development of an *in vivo* model to assess human prostate cancer stem cell targeted therapy. (months 30-60)

No work on this aim has been completed to date. Given the difficulty of cultivating primary human prostate cancer cells in vitro/in vivo by us, and virtually investigators in the field, we may consider performing this aim with established cell lines (LNCaP, PC3 and DU-145). In this alternative approach, we may FACS cells by putative stem cell markers (e.g., CD44, CD144, side population) and establish xenografts from each line. Thereafter, we would treat with different therapies (hormone ablation, chemotherapy- as appropriate) once tumors became palpable (.5 cm x .5 cm).

Key Research and Training Accomplishments

Research accomplishments:

- None this past year

Training accomplishments:

- Coursework:
  - Attended local seminars on cell biology, stem cell biology and cancer biology in the Texas Medical Center

- Conferences/journal clubs:
  - Attend weekly cancer stem cell seminar with Jeffrey Rosen PhD at Baylor College of Medicine
  - Continue to meet with mentors Brenner and Logothetis

- Clinical responsibilities
  - Continue Urology clinic and GU Multi-disciplinary clinic
  - Continue operative schedule

Notable happenings: Over the last year our lab relocated to The Methodist Hospital Research Institute, a new state of the art research facility connected to The Methodist Hospital. In this new space, we have 4 lab benches in an area designated for cancer research with dedicated tissue/cell culture areas for our own use as well as access to a modern small animal facility in the same building. Importantly, in this new space we also have ready access to shared research resources. In addition, in 2011 my research associate left our group to join a Pathology residency program. Through Dean Tang, PhD, a collaborator, we were able to identify a former graduate student of his (Hangwen Li, PhD) with expertise and interest in prostate cancer to join our lab as a post-doc.

Reportable Outcomes

1. Manuscript:

   None on this grant in 2011.

2. Employment opportunity:

   Professional: GU oncology section chief of The Methodist Cancer Center; this promotion was facilitated, in part, by my DoD award.

3. Grant funding:

Over the past year, we have been very lucky to get several grants.

09/30/2012-09/29/15 Sreekumar (PI) 10% effort
Department of Defense, Health Disparity Research Award
PC110534 - “Metabolomic Profiling to Distinguish Racially Distinct Prognostic Markers in Prostate Cancer”-contract # pending
The goals of this project are to identify racially distinct novel biomarkers in prostate cancer.
Role: co-PI

12/15/2011-12/14/2012 Grattoni/Palapattu/Khera (PI) 8% effort
Sponsored Research Agreement with NanoMedicalSystems, Inc.
“Nanochannel Technology for the Constant Sustained Delivery of Testosterone from an Implantable Device
The goal of this project is to test and optimize the long term/sustained delivery of testosterone with nanotechnology”
Role: PI; multiple PI (Grattoni, Khera)

9/30/2012- 9/29/2015 Wong (PI) 5% effort
Department of Defense, Prostate Cancer Research Program
PC111860 - “A Label-Free and Chemical-Selective Microendoscope to Enhance Prostate Cancer Surgical Outcomes”
Contract # pending
The goal of this project is to develop and evaluate an entirely new class of non-invasive medical device—a mobile, optical fiber-based, coherent anti-Stokes Raman scattering (CARS) microendoscope—that will allow surgeons to successfully determine precise cancer margins and cavernous nerve location without the use of exogenous labels or contrast agents, during the operation in real-time.
Role: co-PI (S Wong)

UO1 (UCA167234A) Metabolomic Profiling and Biological Basis of Racial Disparity in Prostate Cancer
NCI/NIH
Role: PI; multiple PI grant (Sreekumar/Palapattu)
Council Round: January 2012
We have heard that this grant was selected for funding. The NCI is awaiting a budget before the formal award letter will be sent.

No other reportable outcomes to report.

Conclusion

From the work completed thus far, we conclude that i) 3D culture of human prostate cancer cells with magnetic nanoparticles is not optimal for tumor initiation studies, ii) in vitro co-culture of human prostate cancer cells (established cell lines and primary patient samples) with human prostate fibroblasts hold promise as models of tumor initiation/cancer stem cell activity.

References

1. Souza GR, Molina JR, Raphael RM, et al. Three-dimensional tissue culture based on magnetic cell levitation. Nature nanotechnology;5(4):291-6.

Appendix

None.